

§ 440.10 Benzylpenicilloyl-polylysine concentrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Benzylpenicilloyl-polylysine concentrate is a pale yellow to dark yellow aqueous solution of benzylpenicilloyl *e* substituted poly-L-lysine. It contains one or more suitable and harmless buffers. It is so purified that:

(i) It contains not less than 50 percent and not more than 70 percent benzylpenicilloyl substitution on the polylysine.

(ii) The benzylpenicilloyl concentration is not less than $1.25 \times 10^{-2} M$ and not more than $2.0 \times 10^{-2} M$.

(iii) The penamaldate concentration is not more than $6.0 \times 10^{-4} M$.

(iv) The penicillenate concentration is not more than $2.0 \times 10^{-4} M$.

(v) Its pH is not less than 6.5 and not more than 8.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for percent benzylpenicilloyl substitution, benzylpenicilloyl content, penamaldate content, penicillenate content, and pH.

(ii) Samples required: 2 vials, each containing not less than 5 milliliters.

(b) *Tests and methods of assay*—(1) *Percent benzylpenicilloyl substitution*—(i) *Lysine content*—(a) *Equipment.* Amino acid analyzer capable of:

(1) Separating the hydrolysis products of benzylpenicilloyl polylysine into discrete components by means of an ion-exchange column.

(2) Mixing the separated amino acid components with a ninhydrin reagent and promoting the reaction in a coil at elevated temperatures.

(3) Quantitating the ninhydrin positive materials by means of a suitable colorimeter and recorder.

(b) *Reagents*—(1) Citrate buffer: Dissolve and dilute 19.69 grams of sodium citrate dihydrate, 16.5 milliliters of hydrochloric acid, 0.1 milliliter of pentachlorophenol, 5 milliliters of thiodiglycol in 900 milliliters of dis-

tilled water; adjust to a pH of 2.2 and dilute to 1 liter with distilled water.

(2) Calibration mixture: Dissolve and dilute equal molar amounts of ammonia, and the L form of lysine in the citrate buffer to result in final concentrations of $2.5 \times 10^{-4} M$ for each.

(c) *Preparation of standard and sample solutions*—(1) *Standard solution* (standard lysine solution ($2.5 \times 10^{-4} M$)). Transfer an accurately weighed portion of 54.8 milligrams of lysine dihydrochloride to a 100-milliliter volumetric flask. Dissolve and dilute to mark with citrate buffer. Make an accurate tenfold dilution of this solution with citrate buffer. The resulting standard solution is $2.5 \times 10^{-4} M$ with respect to lysine.

(2) *Sample solution.* Dilute 1 milliliter of the benzylpenicilloyl-polylysine concentrate to 10 milliliters with distilled water. Mix 1 milliliter of the diluted solution with 1.5 milliliters of 6.0*N* hydrochloric acid and seal in an ampule under nitrogen. Hydrolyze the solution for 22 hours at 110° C. Transfer the contents of the ampule quantitatively into a 50-milliliter round bottom flask and dry by rotary evaporation. Wash the contents and evaporate to dryness three times using 5-milliliter portions of distilled water. Dissolve the hydrolysate in 10 milliliters of citrate buffer.

(d) *Procedure.* Standardize the procedure for use of the amino acid analyzer with the calibration mixture. Apply 0.5 milliliter of the lysine standard solution to the amino acid analyzer and determine the area of the lysine peak. Apply 0.5 milliliter of the sample solution to the amino acid analyzer and determine the area of the lysine peak.

(e) *Calculations.* Calculate the lysine content by the following formula:

$$\frac{\text{Molar concentration of lysine in the benzylpenicilloyl-polylysine concentrate}}{B \times C} = \frac{A \times 2.5}{B \times C}$$

where:

A=The area of the lysine peak of the sample solution.

B=The area of the lysine peak of the standard solution.

C=The percent purity of the lysine dihydrochloride.

(ii) *Benzylpenicilloyl content*—(a) *Reagents.* (1) Mercuric chloride solution: Dissolve 35 milligrams of mercuric

chloride in 500 milliliters of distilled water.

(2) Saline phosphate buffer, pH 7.6: Dissolve 9 grams of sodium chloride and 1.38 grams monobasic sodium phosphate in 900 milliliters of distilled water, adjust to pH 7.6 and dilute to 1 liter with distilled water.

(b) *Preparation of sample solution.* Transfer 1 milliliter of the benzylpenicilloyl-polylysine concentrate into a 500-milliliter volumetric flask and dilute to volume with saline phosphate buffer, pH 7.6.

(c) *Procedure.* Transfer 3 milliliters of the sample solution into a spectrophotometric cell. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the initial absorbance at 282 nanometers. Thereafter, react the diluted benzylpenicilloyl-polylysine solution with 0.02-milliliter portions of the mercuric chloride solution. Determine the absorbance at 282 nanometers at 1 and 3 minutes after each addition of mercuric chloride solution. The increased absorbance at 282 nanometers is used in calculating the benzylpenicilloyl content. Calculate the benzylpenicilloyl content by means of the following formula:

$$\text{Molar benzylpenicilloyl content} = \frac{(A_1 - A_2) \times 500}{22,325}$$

where:

A_1 =The highest absorbance at 282 nanometers

A_2 =The initial absorbance at 282 nanometers

22,325=The molar absorptivity of the penamaldate formed by the reaction of the benzylpenicilloyl moiety with the mercuric chloride at a pH of 7.6.

Percent benzylpenicilloyl substitution=(Molar benzylpenicilloyl content \times 100)/Molar lysine content

(2) *Penicillenate and penamaldate content.* Dilute 1 milliliter of the benzylpenicilloyl-polylysine concentrate to 50 milliliters with saline phosphate buffer, pH 7.6. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the absorbance at 322 and 282 nanometers. Calculate the penicillenate content by the following formula:

$$\text{Molar penicillenate content} = \frac{\text{Absorbance at 322 nanometers} \times 50}{26,600}$$

where:

26,600=Molar absorptivity of the penicillenate moiety at 322 nanometers at a pH of 7.6

Calculate the penamaldate content by the following formula:

$$\text{Molar penamaldate content} = \frac{\text{Absorbance at 282 nanometers} \times 50}{22,325}$$

where:

22,325=Molar absorptivity of the penamaldate moiety at 282 nanometers at a pH of 7.6.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

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§ 440.11 Carbenicillin indanyl sodium.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Carbenicillin indanyl sodium is the monosodium salt of *N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] hept-6-yl)-2-phenylmalonamic acid, 1-(5-indanyl) ester. It is so purified and dried that:

(i) Its potency is not less than 659 micrograms and not more than 769 micrograms of carbenicillin per milligram on an anhydrous basis at the time of certification, and not less than 630 micrograms of carbenicillin per milligram on an anhydrous basis at any time during the expiration period.

(ii) [Reserved]

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 5.0 nor more than 8.0.

(v) It gives a positive result to the identity test for carbenicillin indanyl sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.